12. The method of claim 1, wherein multiple chambers with ultrafiltration are arranged in parallel with a single mass spectrometer for steps e and f.

A marked up version and clean version of the claims are appended herein.

REMARKS

I. Status of the Claims

Claims 1, 5 and 8 are amended. Claim 12 is added.

Claims 1-12 are pending.

II. The Inventors Submit another Declaration

The examiner requested that the inventors file a substitute Declaration of Inventorship because Dr. Bolton crossed out "United States" and hand-corrected her citizenship to "Canada." The examiner requested a typed correction. The substitute declaration is appended herein.

III. Practicing Claims 9 and 11 Does Not Require "Undue Experimentation"

In claim 9 of the pending patent application, the selection of an ultrafiltration membrane with pore sizes that would allow the "sample molecules to pass through but not the biological material" is disclosed. Support in the specification may be found at least on page 3, lines 18-19; page 7, lines 1-12; page 12, lines 6-14; page 13, lines 25-29. When designing one of the ultrafiltration experiments, the biological material is selected first by the practitioner. For example, if the biological material is an enzyme such as glutathione-S-transferase, then it is known by biochemists that this molecule weighs approximately 30,000. Therefore, an ultrafiltration membrane would be selected with a lower molecular weight cut-off such, *e.g.* 10,000. Next, any test mixture consisting of compounds weighing < 10,000 may be tested. The use of the present invention in this manner is easily accomplished by one skilled in the art and that no undue experimentation is needed.

Claims are directed to compounds that consist of drug mixtures, and the biological material includes cells. Commercially available ultrafiltration membranes have pore sizes with molecular weight cut-offs typically of 1,000, 3,000, 10,000, 30,000, or even 100,000.

One skilled in the art of biochemistry knows that cells, for example, are too large to pass though any of these ultrafiltration membranes. Therefore, the molecular weight cut-off of the ultrafiltration membrane need only be selected to be larger than the drug mixture being screened.

When screening compound mixtures for potential new drugs or lead compounds, one skilled in the art would know the molecular weight range of the compounds in the mixture. Typically, practitioners of drug discovery screen combinatorial libraries containing known compounds of defined molecular weights. Therefore, selection of an appropriate pore size membrane would be a trivial matter. Furthermore, because most drugs weigh less than 1,000, any of these membranes would be satisfactory for most applications.

If the test kit of claim 11 would contain cells as the biological material, an ultrafiltration membrane would be selected by those of skill in the art which would allow virtually all drug-like molecules to pass through, but not cells (for example, a molecular weight cut-off of 30,000) to passthrough. Also, a buffer would be known to those of skill in the art that is suitable for use with cells. Standard solutions of test compounds, and standard compounds, would be provided that would be ready to use with cells. No preliminary experimentation would be required to use this kit.

Therefore, applicants' disagree with the rejection, "Claims 9 and 11 are rejected under 35 U.S.C. 112, first paragraph."

Commercial suppliers of ultrafiltration membranes such as Millipore (Amicon brand name) use MWCO (molecular weight cut-off) and not cm or mm to describe the pore size of their products. Therefore, applicants' disagree with item 6, "membrane pore size was not specified in standard language."

Applicants' disagree with item 7 of the Office Action which states that no reference was made in the claims or specification to specific components of the kit so that one skilled in the art would not be able to make and use this kit. The essential components for this kit are listed in claim 11 and consist of an ultrafiltration membrane, a solution containing a biological material, a buffer, a test solution, and a set of standard solutions of predetermined characteristics. Claim 11 was written to include a variety of different biological materials and test solutions. Choosing cells as the biological material and drug mixtures as the test mixture, the kit would be easy to prepare by someone skilled in the art. For example, the ultrafiltration membrane must have a MWCO > 1,000 to allow all drug-like molecules to pass through (for example, 30,000 MWCO would be satisfactory for all cases). Cells (such as the example of Caco-2 cells exemplified in

the patent application), could not pass through an ultrafiltration membrane with a MWCO of 30,000. The selection of buffer would also be obvious to someone skilled in the art, because the buffer must be compatible with the cells.

Please remove rejections under 35 U.S.C. § 112, 1st paragraph.

IV. Terms in Claims 1, 5, 6, 7, 8, 9, 10 and 11 Are Clear

Claims 1 and 5-11 are rejected under 35 U.S.C. § 112, 2nd paragraph.

Contrary to the examiner's opinion, terms in these claims are clear. The title of the application specifies the subject matter to be "screening of xenobiotics and endogenous compounds for metabolic transformation, formation of toxic metabolites, and bioavailability." Claim 1 summarizes these attributes of metabolic transformation, formation of toxic metabolites and bioavailability as "predetermined characteristics" (see page 2, lines 9-14). Furthermore, claim 6 further defines these predetermined characteristics as "functioning as a substrate for an enzyme, showing desirable rates of enzymatic catalysis, showing desirable rates of cell permeability or transport, showing enzymatic activation to reactive or toxic metabolites" (see support on page 3, lines 7-10 of the specification). Therefore, the nature and subject matter of the invention are clearly and completely stated in the title and the claims.

Regarding "predetermined characteristics" defined in claim 6, the examiner questions the term "predetermined" because "they were not physically, genetically or biochemically altered or manipulated" (Action, page 6). When screening drug-like molecules during the process of drug discovery and development, the goal is to find drugs that show high pharmacological activity, low toxicity, high bioavailability, high gut permeability, low first-pass hepatic metabolism, water solubility, and so forth. For specific pharmacological applications, all of these features are "predetermined characteristics." In other words, the potential new drugs being developed by researchers must meet certain criteria or "predetermined characteristics." Only those compounds that meet these criteria will be selected for clinical trials in humans. The essence of the present invention is to streamline and accelerate the process of identifying the drug candidates possessing these characteristics.

Claim 5 (not claim 11) is amended to read, "compounds and their metabolites"

The examiner asks for clarification of the concurrent use of the terms, "reactive" and "toxic" in claim 6. The invention is a means of screening compound for metabolic activation to reactive metabolites. In humans, reactive metabolites are toxic to cells and organs and can cause harm and even death. However, not all reactive metabolites are toxic at low doses, because humans have defense mechanisms that protect cells and organs from reactive molecules. Therefore, both terms are used because the physiological significance of reactive metabolites is toxicity.

The examiner questions "suitable conditions" in claim 1. Support for this term is found in the specification at least on page 3, lines 12-17. If the biological material is defined as cells, suitable conditions for their use would include the well known factors such as physiological temperature of 37°C and atmospheric oxygen and carbon dioxide levels. The time required for the interaction of cells and the drug mixture will be determined by the flow rate of buffer through the ultrafiltration chamber. In the examples given in the specification the flow rate is typically 70-100 mL/min. This flow rate should be adequate for all cell experiments. If not, then lowering the flow rate to slightly lower values such as 20-50 mL/min should solve potential problems. The remaining variables are the concentration of the drug mixture and the number of cells per mL in the ultrafiltration chamber.

The number of cells in an experiment depends upon the application. In the case of measuring cell permeability, the number of cells used would be 5 to 10 million per mL as shown in the examples in the specification. In the case of using cells for studies of metabolic activation or metabolism, as few as 100,000 cells per mL would be adequate for most applications. Fewer cells would probably still be sufficient if the flow rate of buffer through the chamber were reduced. More cells would also work. In practice, the optimum number of cells for a particular application could be determined by routine experimentation. However, a wide range of cell numbers would still work.

The concentration of the drug mixture should be as low as possible but still easily detected using analytical methods such as absorbance, fluorescence or mass spectrometry. Ideally, the drug concentration should be so low as to produce no cytotoxicity and not saturate receptors, transport proteins, or drug metabolizing enzymes. Because the interactions with

receptors, enzymes and proteins are usually poorly defined early in the drug discovery and development process, the practical means to determine the drug level to be used in an experiment will be the analytical detection limit which is easily determined prior to the experiment by those of skill in the art.

V. Claim 10 is not Anticipated

Metzger *et al.* and Stevanovic describe the use of mass spectrometry for analyzing peptide mixtures but do not anticipate claim 10. Mass spectrometry is recognized in the literature as a useful tool for the analysis of peptide libraries. However, the present invention is not restricted to the analysis of peptides. Instead, any mixture of drugs and/or drug metabolites, drug-like molecules and/or their metabolites may be analyzed using the present invention.

More importantly for the present invention, mass spectrometry is a valuable detector for use *on-line* with the ultrafiltration system of the present invention. In Metzger *et al.* and Stevanovic *et al.*, mass spectrometry is used to analyze peptide mixtures, but the origin of those mixtures is not relevant nor is the amount of time that has elapsed since the peptide mixtures were generated. In the present invention, the compounds are analyzed *in real time* as they are formed and elute from the ultrafiltration chamber. This temporal aspect is critical to increasing the throughput of analysis as well as minimizing any degradation of the mixture that might occur if the samples were simply collected and analyzed later.

VI. Claim 1 is Not Obvious Over Weiboldt *et al.*

The examiner cites only one publication to support a rejection under 35 U.S.C. § 103(a).

The publication does not teach all the elements of amended claim 1, therefore it does not make claim 1 obvious.

Weiboldt *et al.* describes a screening method for drug discovery using ultrafiltration and mass spectrometry. However, the present invention is not a method for drug discovery, therefore Weiboldt *et al.* is not relevant prior art. Instead of a drug discovery method, the present invention is intended for screening for "metabolic transformation, formation of toxic metabolites, and bioavailability." See also page 1, lines 19-22, "drug metabolism and toxicity become the new bottleneck to the process of bringing new drugs to market." Furthermore, Weiboldt *et al.* describe the use of an antibody (a large protein) as the "biologic material." In the present

invention, the biological material includes not only a protein but "a peptide, an oligonucleotide, an oligosaccharide, a microsome, a cell, a tissue, an enzyme, a receptor, DNA and RNA."

In the present application, the use of cells, for example, is not for affinity purification as described by Weiboldt. Instead, the intact cellular membrane is used for studies of cell permeability, and cellular enzymes and cofactors for studies of metabolism and metabolic activation. These unusual applications are not described by Weiboldt *et al.* and are not obvious to one skilled in the art. They are novel and unique to the present patent application. Moreover, Weiboldt's method **cannot** provide a "continuous flow" as in claim 1 (b) because Weiboldt uses centrifugal ultrafiltration which has discrete stops.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck*, 947 F,2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). See MPEP § 2143- § 2144.09 for decisions pertinent to each of these criteria.

To properly combine references to reach a conclusion of obviousness, there must be some teaching, suggestion or inference in either, or all of the references, or knowledge generally available to one skilled in the art, which would have led one to combine the relevant teachings of the references. *Ashland Oil, Inc. v. Delta Resins and Refractories, Inc. et al.* (CAFC 1985) 776 F. 2d 281, 227 USPQ 657; *Ex parte Levengood, supra.* Both the suggestion to make the claimed composition or device or carry out the claimed process and the reasonable expectation of success must be founded in the prior art, not in applicant's disclosure. *In re Vaeck* (CAFC 1991) 947 F. 2d 488, 20 PQ. 2d 1438. Citing references which merely indicate that isolated elements and/or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious, *Ex parte Hiyamizu* (BPAI 1988) 10 PQ. 2d 1393, absent evidence of a motivating force which would impel persons skilled in the art

to do what applicant has done. *Ex parte Levengood* (BPAI 1993) 28 PQ. 2d 1300. The references, viewed by themselves and not in retrospect, must suggest doing what applicant has done. *In re Shaffer* (CCPA 1956) 229 F. 2d 476, 108 USPQ 326; *In re Skoll* (CCPA 1975) 523 F. 2d 1392, 187 USPQ 481. The examiner is using legally unacceptable hindsight as the basis for his rejections.

VI. Substitute FIGS. 1-7 Are Appended

Substitute FIGS. 1-7 are appended that are identical to the figures in the priority provisional application and are described fully in the "Brief Description of the Drawings" and the "Detailed Description of the Invention" of the pending application. No new matter is added.

VII. Summary and Conclusion

For the reasons stated above, applicant requests allowance of all pending claims.

Please contact applicant's representative if you have any questions.

No other fees are believed due at this time, however, please charge any deficiencies or credit any overpayments to deposit account number 10-0435 with reference to our attorney docket number (21726/90386)

Respectfully submitted,

Alice O. Martin
Registration No. 35,601

Barnes & Thornburg
2600 Chase Plaza
10 South LaSalle Street
Chicago, Illinois 60606
(312) 214-8316
June 5, 2001
CHDS01 AOM 101617v1

CLEAN COPY OF PENDING CLAIMS
SERIAL NO. 09/471,523

WE CLAIM

1. A method for determining whether a compound from a sample has predetermined characteristics that would make it suitable for a specific purpose, said method comprising:

a. obtaining a biological material in a first solution or suspension;

b. maintaining a continuous flow of a supportive solution through the first solution or suspension;

c. adding the sample to the continuous flow of the supportive solution;

d. reacting the biological material in the first solution or suspension with the compound in the sample to provide metabolites, or to assess permeability and bioavailability;

e. washing the results of the reacting between the biological material in the first solution and the compound in the sample through an ultrafiltration membrane to form a second solution; and

f. analyzing the second solution to determine whether the compound in the sample has the predetermined characteristics.

2. The method of claim 1, wherein the biological material is selected from a group consisting of a protein, a peptide, an oligonucleotide, an oligosaccharide, a microsome, a cell, a tissue, an enzyme, a receptor, DNA and RNA.

3. The method of claim 1, wherein the compound is selected from the group consisting of a natural product, a combinatorial library, a drug, a drug mixture, a xenobiotic compound, a mixture of xenobiotic compounds, an endogenous compound, and a mixture of endogenous compounds.

4. The method of claim 1, wherein the supportive solution is selected from a group consisting of a buffer, a nutrient medium, or a combination thereof, said supportive solution capable of maintaining the biological material in a state wherein the biological material can interact with a compound in the sample.

5. The method of claim 1, wherein the continuous flow facilitates the reacting of the biological material with the sample in the first solution or suspension and facilitates the removal

of compounds and their metabolites from the sample [and their metabolites] by washing them through the ultrafiltration chamber into the second solution.

6. The method of claim 1, wherein the predetermined characteristics consist of functioning as a substrate for an enzyme, showing desirable rates of enzymatic catalysis, showing desirable rates of cell permeability or transport, showing enzymatic activation to reactive or toxic metabolites.

7. The method of claim 1, wherein the sample is added to the continuous flow by means of injection.

8. The method of claim 1, wherein the suitable conditions for reacting of the biological material in the first solution with the compound in the sample comprises mixing the sample with the biological material to achieve a homogeneous distribution of sample, temperature control to maintain function of the biological material, adequate concentration of sample and sufficient amount of biological material to facilitate analysis, sufficient time for interaction, control of atmospheric gases (oxygen and carbon dioxide) to maintain function of the biological material.

9. The method of claim 1, wherein the ultrafiltration membrane has pore sizes that allow the sample molecules to pass through but not the biological material.

10. The method of claim 1, whereas the analyzing of the second solution is by mass spectrometry.

11. A kit for analyzing compounds in a sample, said kit comprising in separate containers, an ultrafiltration membrane, a first solution containing a biological material, a buffer, a test solution, and a set of standard solutions with predetermined characteristics.

12. The method of claim 1, wherein multiple chambers with ultrafiltration are arranged in parallel with a single mass spectrometer for steps e and f.

MARKED UP VERSION OF PENDING CLAIMS
SERIAL NO. 09/471,523

WE CLAIM

1. (Amended) A method for determining whether a compound from a sample has predetermined characteristics that would make it suitable for a specific purpose, said method comprising:

a. obtaining a biological material in a first solution or suspension;

b. maintaining a continuous flow of a supportive solution through the first solution or suspension;

c. adding the sample to the continuous flow of the supportive solution;

d. [providing suitable conditions for the interaction of] reacting the biological material in the first solution or suspension with the compound in the sample to provide metabolites, or to assess permeability and bioavailability;

e. washing the results of the [interaction] reacting between the biological material in the first solution and the compound in the sample through an ultrafiltration membrane to form a second solution; and

f. analyzing the second solution to determine whether the compound in the sample has the predetermined characteristics.

2. The method of claim 1, wherein the biological material is selected from a group consisting of a protein, a peptide, an oligonucleotide, an oligosaccharide, a microsome, a cell, a tissue, an enzyme, a receptor, DNA and RNA.

3. The method of claim 1, wherein the compound is selected from the group consisting of a natural product, a combinatorial library, a drug, a drug mixture, a xenobiotic compound, a mixture of xenobiotic compounds, an endogenous compound, and a mixture of endogenous compounds.

4. The method of claim 1, wherein the supportive solution ~~is~~ selected from a group consisting of a buffer, a nutrient medium, or a combination thereof, said supportive solution capable of maintaining the biological material in a state wherein the biological material can interact with a compound in the sample.

MARKED UP VERSION OF PENDING CLAIMS
SERIAL NO. 09/471,523

5. (Amended) The method of claim 1, wherein the continuous flow facilitates the reacting [interaction] of the biological material with the sample in the first solution or suspension and facilitates the removal of compounds and their metabolites from the sample [and their metabolites] by washing them through the ultrafiltration chamber into the second solution.

6. The method of claim 1, wherein the predetermined characteristics consist of functioning as a substrate for an enzyme, showing desirable rates of enzymatic catalysis, showing desirable rates of cell permeability or transport, showing enzymatic activation to reactive or toxic metabolites.

7. The method of claim 1, wherein the sample is added to the continuous flow by means of injection.

8. (Amended) The method of claim 1, wherein the suitable conditions for [interaction] reacting of the biological material in the first solution with the compound in the sample comprises mixing the sample with the biological material to achieve a homogeneous distribution of sample, temperature control to maintain function of the biological material, adequate concentration of sample and sufficient amount of biological material to facilitate analysis, sufficient time for interaction, control of atmospheric gases (oxygen and carbon dioxide) to maintain function of the biological material.

9. The method of claim 1, wherein the ultrafiltration membrane has pore sizes that allow the sample molecules to pass through but not the biological material.

10. The method of claim 1, whereas the analyzing of the second solution is by mass spectrometry.

11. A kit for analyzing compounds in a sample, said kit comprising in separate containers, an ultrafiltration membrane, a first solution containing a biological material, a buffer, a test solution, and a set of standard solutions with predetermined characteristics.

12. The method of claim 1, wherein multiple ultrafiltration chambers are arranged in parallel with a single mass spectrometer for steps e and f.